



## History of the race structure of *Orobanche cumana* and the breeding of sunflower for resistance to this parasitic weed: A review

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### Abstract

Broomrape, caused by *Orobanche cumana*, has affected sunflowers since the early 20<sup>th</sup> century in Eastern Europe. Currently, it limits sunflower oil production in Southern and Eastern Europe and in some areas of Asia, causing around 50% seed losses when susceptible hybrids are grown. Covered in this review are aspects such as: biological processes that are common to *Orobanche* spp. and/or particular to *O. cumana* in sunflower, genetic resistance and its mechanisms, races of the parasite identified in different countries throughout the time and their increasing virulence, and breeding for resistance to some herbicides as a novel control option. The main purpose is to present an updated and, as far as possible, complete picture of the way both the parasitic weed and its host crop have evolved in time, and how they co-exist in the current agriculture. Additionally, we propose a system for determining the races of the parasite that can be internationally adopted from now. In the context of minimal harmful effects on the environment, changing patterns of land use in farming systems, and global environment changes, the final goal of this work is to provide all those interested in parasites from field crops and their integrated management compiled information on the sunflower – *O. cumana* system as a case study.

**Additional key words:** genes of resistance; *Helianthus annuus* L.; broomrape; parasite races; virulence.

**Abbreviations used:** AHAS (acetohydroxyacid synthase); GS (germination stimulants); HR (herbicide resistant); IMI (resistance to imidazolinone); PG (polygalacturonases); PME (pectin methyl esterase); POB (pyrimidyloxybenzoates); QTL (quantitative trait loci); SU (sulfonylurea); TZ (triazolopyrimidines).

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### Sunflower and broomrape as a threat to production

Sunflower (*Helianthus annuus* L.) is the most important oleaginous field crop in Southern and Eastern Europe and, together with olives, its seed is the main source of vegetable oil in the Mediterranean area. Besides Southern and Eastern Europe, the area devoted to sunflower is located in Argentina and China. A total of 26·10<sup>6</sup> ha was cropped to sunflower in the world in 2013, with a production of 45·10<sup>6</sup> tons (<http://faostat3.fao.org/browse/Q/QC/E>). The first ten producers of

sunflower seed for oil extraction account for 85% of the total production. Among them, Ukraine, the Russian Federation and Argentina are leaders with 25, 24, and 7% respectively of the total seed production. The second group of world producers is composed of China, Romania, Bulgaria, France, Turkey, Hungary and Spain which account for the 27% of the world total sunflower seed.

The parasitic plant *Orobanche cumana* Wallr. is the most important biotic constraint to the production of sunflower seed in all the countries where sunflower is grown except in North and South America. The parasit-

ism of *O. cumana* on sunflower dates back to the first half of the 19<sup>th</sup> century in Russia, when the plant, which was a parasite to *Artemisia* spp., found *H. annuus* as a more appropriate host (Antonova, 2014). Thereafter, the geographical spread of *O. cumana* in the world followed the same pattern, with a delay of some decades, as the expansion of the crop of sunflowers in time and over different countries.

Sunflower was first grown as an oleaginous crop in the Saratov and Voronezh regions of Russia in the first half of the 19<sup>th</sup> century and, by the late 19<sup>th</sup> and early 20<sup>th</sup> centuries, the area cropped to sunflower in the country increased to become the first source for vegetable oil. It was in Voronev that an important infection of *O. cumana* in sunflowers was first observed in 1866 (Morozov, 1947), and in a few decades *O. cumana* significantly spread all over the crop in the former USSR and threatened the mass production of sunflower oil. The crop expanded to countries such as Moldova and Romania by the end of the 19<sup>th</sup> century (Duca, 2014; Pacureanu, 2014) and later to others like Serbia or Turkey in the first half of the 20<sup>th</sup> century (Bülbül *et al.*, 1991; Miladinovic *et al.*, 2014). Consequently, infections by broomrape became a limiting factor for oil production around the middle of the last century in Bulgaria, Romania, Serbia and Moldova (Batchvarova, 2014; Duca, 2014; Miladinovic *et al.*, 2014; Pacureanu, 2014).

Particularly interesting is the occurrence of infections by *O. cumana* in Spain, where sunflowers, introduced by Spanish explorers, have been grown since the 16<sup>th</sup> century. Sunflower was first used as an ornamental plant and later for edible purposes. For centuries Cuenca, a province located in the centre of Spain, has been the traditional area for growing confectionery sunflower, and *O. cumana* parasitizing these varieties was first reported there in 1958 (Díaz-Celayeta, 1974). The re-introduction of oilseed cultivars from Eastern Europe favoured the enormous crop expansion in central and in southern Spain in the 1970's and early 1980's, and it was not too long before *O. cumana* gained importance as a parasite of both confectionery and oilseed sunflower, the latter being less severely affected than the former (González-Torres *et al.*, 1982).

Currently, *O. cumana* is present in all the countries of Southern Europe and areas around the Black Sea where sunflowers are grown (Antonova, 2014; Batchvarova, 2014; Duca, 2014; Hargitay, 2014; Jestin *et al.*, 2014; Kaya, 2014; Miladinovic *et al.*, 2014; Molinero-Ruiz & Domínguez, 2014; Pacureanu, 2014; Pototskiy, 2014), as well as in North Africa (Amri *et al.*, 2012), Israel (Eizenberg *et al.*, 2004) and China (Baichun *et al.*, 1996; Ma & Jan, 2014; Shi *et al.*, 2015).

*Orobancha cumana* infects the roots of sunflower early in the growing season, obtaining water and inorganic compounds from the host plant through a xylem to xylem contact (Heide-Jorgensen, 2008). Unlike the vast majority of parasitic plants, *Orobancha* spp. also utilizes the carbon fixed by the host plants, which is transferred through phloem continuity between host and parasite (Dörr, 1990). Moreover, *Orobancha*-induced yield reductions are due to competition for organic solutes, while competition for inorganic solutes plays a small role in determining host productivity, and competition for water is a secondary cause of yield reduction (Stewart & Press, 1990). In the case of *O. cumana*-sunflower, the utilization of host photoassimilates by the parasite results in depletion of resources which are necessary for the growth of sunflower and for the optimal development of seeds. Broomrape stems have a long underground developmental stage, emerging aboveground at around flowering-time of sunflower plants (Melero-Vara & Alonso, 1988). By the time the broomrape has emerged, most of the metabolic imbalance has already been produced by the parasite to sunflower. In fact, and as early as three weeks after inoculation with *O. cumana*, a decrease in secondary metabolites is observed in the host upon infection by the parasite (Pérez-Bueno *et al.*, 2014). From the first emergence of broomrape stems onwards, the impact of *O. cumana* on the crop yield is increasingly evident through absent or small sized capitula, a low number and small seeds, and even plant death. Yield reductions due to infection by *O. cumana* depend on several factors, such as the soil infestation level, aggressiveness of the parasite, sunflower genotype, earliness of broomrape emergence and soil depth, among others (Molinero-Ruiz *et al.*, 2009; Jestin *et al.*, 2014). On average, sunflower seed losses caused by broomrape can be quantified at above 50% when susceptible hybrids are grown, and they frequently reach 100% in heavily infested fields (Domínguez, 1996a; Jestin *et al.*, 2014).

## The parasite *O. cumana*

### Overview of its biology and genetics

Despite the fact that *O. cumana* is a major threat to sunflower cultivation from the Mediterranean region to Central Asia, methods for a sustainable control of this pest are scant and most often ineffective. Thus, understanding the biology of the interaction between the parasite and its host is a necessary step towards the development of selective control methods. This aim should include notably deciphering the physiological

and molecular mechanisms governing the parasite development and the establishment of the interaction with its host. Unfortunately, compared with other plant pathogens such as bacteria and fungi, our knowledge of broomrape biology is limited and comes from studies mainly made on *Phelipanche ramosa*, *Phelipanche aegyptiaca*, and *Orobanche minor*, three other noxious parasitic weeds. Thus, throughout this section the generic name “orobanche” will be used when referring to broomrape species and most of the biological events mentioned below could be applied to *O. cumana*.

*Orobanche cumana* belongs to the *Orobanche* section (synonym of the *Osproleon* section) from the *Orobanchaeae* class of the Orobanchaceae family. *Orobanche cumana* is a close relative of *O. cernua*, two different *Orobanche* species that have long been considered as a single one (Román *et al.*, 2003). In terms of genetics and genomics, the diploid *O. cumana* species, exhibits a chromosome number (2n) of 38 and an estimated genome size of 1.42 Gb (Piednoël *et al.*, 2012). To date, neither the reference transcriptome nor the complete genome sequence are available for this species. Only Expressed Sequence Tags from *Phelipanche aegyptiaca* are publicly available in the Parasitic Plant Genome Project website (PPGP, 2015).

Like any other holoparasite species, broomrapes are achlorophyllous root parasitic plants relying on a host for their nutrition and survival. Orobanche seeds are very small, 200-300 µm, and a large amount of seeds is produced annually, since only one plant can produce several tens of thousands of seeds. These seeds are easily disseminated by wind, water and other agents; for instance, contaminated crop seed lots of sunflower achenes (Castejon *et al.*, 1991). Seed maturation and desiccation are phases of varying durations depending on the species, and environmental factors such as temperature. After release from the flower's capsule, seeds are buried in the soil, where they remain viable in dormancy for decades (Prider *et al.*, 2013). Among the remarkable morphological and physiological adaptations that characterize the orobanche species, the requirement for the seeds to perceive molecules produced by host roots to germinate is probably the most intriguing one. Indeed, the germination of broomrapes is a two-step process corresponding, first, to a conditioning period, thought to be required for the acquisition of their sensitivity to the germination stimulants (GS), followed by the chemical stimulation of the germination itself that ends with the radicle protrusion (Lechat *et al.*, 2012). During the conditioning period, broomrape seeds are exposed to a moist environment and suitable temperatures ranging from 15 to 25°C depending on the species (Linke *et al.*, 1989). If conditions are not favourable for the germination or if seeds are

not stimulated by host roots, seeds enter into secondary dormancy (Matusova *et al.*, 2004). In *P. ramosa* and *O. cumana*, it has been demonstrated that seeds need a minimum 4 days conditioning period at 21°C, during which a global DNA demethylation occurs, which is an epigenetic process required for the seed to become responsive to GS (Lechat *et al.*, 2015). Once conditioned, broomrape seeds are still unable to germinate without stimulation by GS, chemicals compounds produced and exuded in the rhizosphere by surrounding host roots; this mechanism implies that the parasite is in the vicinity of the roots (3-5 mm) on which it will attach itself. The efficient GS for the germination of *O. cumana* seeds, which are present in sunflower root exudates, are the sesquiterpene lactones (Joel *et al.*, 2011; Raupp & Spring, 2013), heliolactone (Ueno *et al.*, 2014), and strigolactones (Yoneyama *et al.*, 2011). Whatever the GS, it has been demonstrated that *O. cumana* seeds respond by an up-regulation of CY-P707A1, an abscisic acid catabolic gene, that then triggers the germination process (Delavault *et al.*, 2013).

During germination, the seed produces a radicle also called procaulome or germ tube of a few mm long that grows towards the host root probably thanks to a positive chemotropism. Seeds of *P. ramosa* and *O. cumana* exposed to root exudates of tobacco and sunflower, respectively, released indole-3-acetic acid even before development of the germ tube suggesting that auxin may be involved in the germination process (Slavov *et al.*, 2004). When the procaulome of *O. cumana* reaches the host root, its apical cells differentiate into papillae producing a carbohydrate that allows parasite adhesion to the root (Joel & Losner-Goshen, 1994). This step of the broomrape life cycle constitutes the transition to parasitic phase. During parasite attachment, the germ tube produces a swollen structure at the apex, the appressorium also called pre-haustorium, which produces intrusive cells, some of which establish connections with the host vessel system. Penetration of the parasite into host root tissues toward sap-conducting vessels involves mechanical and enzymatic processes (Joel & Losner-Goshen, 1993). Intrusive cells progress between host cells that are pushed away under mechanical pressure. To facilitate its progression between host cells, the parasite also degrades the middle lamella of their cell wall. For instance, pectin degrading enzymes such as pectin methyl esterase (PME), polygalacturonases (PG), and rhamnogalacturonases are secreted by germinating seeds of *P. aegyptiaca* and *P. ramosa* (Ben-Hod *et al.*, 1993; Véronesi *et al.*, 2007). Using immunocytochemical methods, Losner-Goshen *et al.* (1998) detected PME in the cytoplasm and cell walls of *O. cumana* intrusive cells localized at the

sunflower-parasite interface. The virulence of the parasite seems to be correlated with the production of these enzymes. Indeed, the intensity of these pectinolytic activities, PME and PG secreted by different races of *O. cumana*, was proportional to the virulence of each race (Véronési *et al.*, 2005).

Once the appressorium penetrates the endodermis, parasite cells progress towards host vessels and then initiate a specialized endophytic organ called haustorium that proceeds to the establishment of vascular connections (Hibberd & Jeschke, 2001). The haustorium is also the transfer structure that enables the parasite to uptake water and nutrients (carbohydrates and nitrogen compounds) from host conductive system. Whereas host factors for the haustorium development (haustorium-inducing factors or HIF) have been characterized in several Orobanchaceae species, those factors have not yet been evidenced in *Phelipanche* and *Orobanche* species (Yang *et al.*, 2015). Histological studies at the *O. cumana*-sunflower interface confirmed the existence of a direct connection between host and parasite xylem elements thanks to parasite cells differentiating into tracheid-like vessels (Labrousse, 2002). Moreover, some parasite cells were observed to be in close contact with phloem cells in host roots, suggesting that *Orobanche* are “phloem feeders”. In fact, when the phloem-mobile symplasmic tracer carboxyfluorescein is applied on source host-leaves, the molecule is translocated in a few minutes into the parasite demonstrating unequivocally the existence of a symplasmic continuum between host and parasitic phloem (Péron, 2010). Penetration of the intrusive cells into the xylem triggers their division leading to the formation of a tubercle, a callus-shaped bulge of parasite cells outside the root. Once vascular connections are established and the haustorium is functional, the parasite then acts as a supernumerary sink organ for the host plant by expressing genes involved in sucrose metabolism and the establishment of a strong sink strength (Draie *et al.*, 2011; Péron *et al.*, 2012). The attached parasite diverts hormones and photoassimilates from the host plant, mainly auxin and sucrose (Bar-Nun *et al.*, 2008; Abbes *et al.*, 2009). The tubercle, with a diameter of 0.5 to 2.5 cm, serves as a transient storage organ and develops numerous adventitious roots; the apical bud of this tubercle gives rise to a subterranean shoot and then to a flowering spike, after emergence from the soil, that rapidly produces a large amount of tiny seeds.

Thus, much remains to be studied of the biology of these intriguing interactions, especially that between *O. cumana* and its host, to identify vulnerabilities that would be specific targets for new control methods.

## Race structure of *O. cumana*

The first mention to different races within the *O. cumana* species dates back to the beginning of the 20<sup>th</sup> century. Efforts made by Soviet breeders at the Saratov experimental station resulted, by 1920, in the release of genetically resistant varieties of sunflower. Since these varieties effectively controlled the races of *O. cumana* from the Saratov and Voronezh regions but not those affecting sunflowers in the Rostov and Krasnodar regions, two races, namely A and B, were differentiated in Russia from then on. Both races had a different geographical distribution: race A was common in Saratov and Voronezh regions and race B was found in Rostov and Krasnodar regions (Antonova, 2014). Also in Moldova and Ukraine (former USSR) race B of *O. cumana* was identified in the 1930's (Duca, 2014; Miladinovic *et al.*, 2014). The control provided by sunflower hybrids with resistance to race B was overcome by race C of *O. cumana* in Moldova in the 1970's (Duca, 2014). Thereafter, breeding works resulted in new varieties of sunflower with resistance to this race allowing an efficient control of the parasite in the former USSR until the 1990's. Throughout the past decades a new failure in the genetic control of *O. cumana* has occurred in the Russian Federation as a consequence of crop intensification and short crop rotations, together with the use of genetic material from foreign breeding programmes which was therefore susceptible to the local races of the parasite (Antonova, 2014). Recently, new and highly virulent races of *O. cumana* from the southern regions of the Russian Federation have been identified as races E, F, G and H (Antonova *et al.*, 2013). The presence of races E and F of the parasite has also been recently reported in the Republic of Moldova (Gisca *et al.*, 2013; Duca, 2014), as well as races C and G in Kazakhstan (Antonova, 2014). In Ukraine, the increase in the area cropped to sunflower throughout the last decade, together with short rotations, has been associated with the frequent occurrence of race E followed by races F and G in the south and southeast of the country (Pototskyi, 2014).

*Orobanche cumana* was reported to be affecting sunflower in Romania in early 1940's (Pacureanu, 2014). The first attempt to determine races of the parasite in that country was that of Vrânceanu *et al.* (1980), who used a set of sunflower genotypes, each of them carrying one single major gene of resistance. These genes ( $Or_1$  to  $Or_3$ ) were reported to control races A to E respectively in Romania, each of them also conferring resistance to the previously described races (what is termed as cumulative resistance) (Vrânceanu *et al.*, 1980). As occurred in other countries, races A to E in Romania were effectively controlled through ge-

netic resistance for some decades, until race F was identified in the mid-nineties (Pacureanu-Joita *et al.*, 2008).

The first report of *O. cumana* in Serbia dates back to the 1950's; the populations detected were probably race B, because they were controlled by Russian oil varieties and hybrids with resistance to this race (Miladinovic *et al.*, 2014). Economically important incidences of broomrape on sunflower were again observed in that country in the 1990's and identified as a race E (Mihaljčević, 1996). During the last 20 years, and even though the parasite being spread to new sunflower production areas in the country, no new races have occurred so far (Miladinovic *et al.*, 2014). Infections of sunflowers by *O. cumana* were reported in Bulgaria in the first half of the 20<sup>th</sup> century, when the parasite populations were characterized as races A and B. Some decades later, races C, D and E, were identified and thereafter effectively controlled by means of genetic resistance. Race E of *O. cumana* remained predominant in Bulgaria in the first years of the 21<sup>st</sup> century until the sunflower oil production of the country was threatened again by race F (Shindrova, 2006). Nowadays, races E, F and possibly race G are widely spread in the sunflower growing areas of Bulgaria (Batchvarova, 2014).

With respect to other sunflower growing countries, in Hungary, populations of *O. cumana* were characterised as being low to moderately virulent (races A–D) (Zoltán, 2001). Currently, race E is the most frequent one in the country (Hargitay, 2014) although race F has also been identified (Molinero-Ruiz *et al.*, 2014). Turkey is another country where infections of sunflowers by *O. cumana* endanger the crop production in every growing season. Race F is widely distributed in Trakya region (Kaya *et al.*, 2004), which accounts for more than half of the total area of sunflowers in the country. Also, race G has been identified in several locations of this same region (Molinero-Ruiz *et al.*, 2014). In the past few years, infections of sunflower hybrids by *O. cumana* have also become frequent in the Anatolia region, but the race/s of the parasite have not been determined (Kaya *et al.*, 2012). The presence of *O. cumana* parasitizing sunflower in France was not reported until 2007 (Jestin, 2012). It is currently located mainly in the south and also in the west of the country (Jestin *et al.*, 2014), although no information about race/s is available. *Orobanche cumana* race A was identified in the northeast of China in the 1990's (Baichun *et al.*, 1996). A widespread increase in new virulent parasite races has occurred since then, so that the wide distribution of races A to F there, as well as the identification of race G in Inner Mongolia, have recently been reported (Ma & Jan, 2014; Shi *et al.*, 2015).

Spain is the country where the greatest efforts have been devoted to characterizing races of *O. cumana* during the past 40 years. Race B of the parasite was prevalent in Spain (Saavedra Del Río *et al.*, 1994) until race C was detected in the 1970's (González-Torres *et al.*, 1982), when an unexpectedly high infection of *O. cumana* occurred in the oilseed sunflower variety Peredovik, which was obtained by Soviet breeders as being resistant to race B. Interestingly, populations of *O. cumana* race B from Spain did not fit the cumulative resistance of the differentials proposed in Romania (Melero-Vara *et al.*, 1989) as would occur years later, when the inbred line L86, registered as resistant to race F (Fernández-Martínez *et al.*, 2004), was extremely susceptible to the less virulent race E (Molinero-Ruiz *et al.*, 2006). Also interesting about races of *O. cumana* in Spain is that in the 1980's two different races of the parasite were identified, one in the south and another in the centre of the country, and characterized as races D and E, respectively (Melero-Vara *et al.*, 1989). Pathogenic and molecular differentiation of *O. cumana* populations from Spain as related to geographical origin were again recently reported (Molinero-Ruiz *et al.*, 2006; 2014; Pineda-Martos *et al.*, 2013). The last outbreak of genetic resistance in the crop in Spain occurred twenty years ago after the identification of race F (Saavedra del Río *et al.*, 1994; Alonso *et al.*, 1996). During the early years of 2000, *O. cumana* race F became widely distributed in southern Spain (Molinero-Ruiz *et al.*, 2006; 2009) and is also present in some sites of northern and central Spain (Fernández-Escobar *et al.*, 2008; Molinero-Ruiz & Dominguez, 2014).

Important efforts of breeders have been devoted to the search for effective resistance against the increasingly virulent parasite populations and, as a result, resistant genotypes have been obtained and released (see 'Sunflower breeding and genetics of resistance to *O. cumana*' section). In each country, sunflower material identified as being resistant to *O. cumana* has been used to differentiate local races of increasing virulence and termed from A to E, F or G. Because no comparative studies have been conducted to test the correspondence of races between countries, an unbundled knowledge of pathogenic traits of the parasite is currently occurring. In spite of races A to G already having been identified in many countries (Table 1), very few works assess the similarity of those populations from different geographic origins and characterized as belonging to the same race (Molinero-Ruiz *et al.*, 2014).

When determining races of crop pathogens, a long list of terms is frequently used to designate a wide range of races, making the nomenclature system extremely complex. When this happens, mathematical

codes are, by far, more advantageous than the use of consecutive numbers or letters given in chronological order of race discovery. To ease communication and the comparisons of results of race characterization, a universal adoption of the coded triplets system (Limpert & Müller, 1994) is frequent for many plant pathogens (Liebenberg & Pretorius, 2011; Gurung *et al.*, 2013; Dreiseitl, 2014) including *Plasmopara halstedii* Farl. Berl. & de Toni, which causes sunflower downy mildew (Molinero-Ruiz *et al.*, 2002). Due to: a) the diversity of races of *O. cumana* identified worldwide,

and b) their characterization according to the reaction of genes of resistance in sunflower genotypes which are particular to each crop region, the use of the coded triplets system as a simple and global method to internationally determine the races of the parasite seems imperative at this moment. In this review we propose that this system be internationally adopted from now on by sunflower breeders and pathologists. The essential component of the coded triplets system is a group of sunflower lines, termed differentials, which are divided into sets of up to three lines each. In Table 2 we

**Table 1.** Races of *Orobanche cumana* identified –in the past and at present– in several countries where sunflowers are grown and parasite infections are known to occur

Country	Races of <i>O. cumana</i> identified <sup>a</sup>		References
	Past	Present	
Bulgaria	A, B, C, D, E	E, F, G	Shindrova, 2006; Batchvarova, 2014
China	A	A, B, C, D, E, F, G	Ma & Jan, 2014; Shi <i>et al.</i> , 2015
France	Not present	NK <sup>b</sup>	Jestin, 2012; Jestin <i>et al.</i> , 2014
Hungary	A, B, C, D	E, F	Zoltán, 2001; Hargitay, 2014; Molinero-Ruiz <i>et al.</i> , 2014
Kazakhstan	NK	C, G	Antonova, 2014
Moldova	B, C	E, F	Gisca <i>et al.</i> , 2013; Duca, 2014
Romania	A, B, C, D, E	F, G	Vrânceanu <i>et al.</i> , 1980; Pacureanu-Joita <i>et al.</i> , 2008; Pacureanu, 2014
Russia	A, B, C, D	D, E, F, G, H	Tolmachyov, 1990; Antonova <i>et al.</i> , 2009; 2013; Antonova, 2014
Serbia	B, E	E	Mihaljčević, 1996; Miladinovic <i>et al.</i> , 2014
Spain	B, C, D, E	E, F	González-Torres <i>et al.</i> , 1982; Melero-Vara <i>et al.</i> , 1989; Saavedra Del Río <i>et al.</i> , 1994; Alonso <i>et al.</i> , 1996; Molinero-Ruiz <i>et al.</i> , 2006; Fernández-Escobar <i>et al.</i> , 2008; Molinero-Ruiz <i>et al.</i> , 2009; Molinero-Ruiz & Domínguez, 2014
Turkey	NK	F, G	Kaya <i>et al.</i> , 2004; 2012; Molinero-Ruiz <i>et al.</i> , 2014
Ukraine	A, B, C, D	E, F, G	Tolmachyov, 1990; Pototskyi, 2014

<sup>a</sup> This review was prepared as a result of the active scientific interface which was stimulated by the Third International Symposium of *Orobanche* spp. of Sunflower, which was held in Córdoba (Spain) in June 2014. Therefore “Present” refers to 2014 and “Past” to earlier decades. <sup>b</sup> NK: not known; the parasite was reported, but no information about race/s is known.

**Table 2.** Proposal for standardized characterization and nomenclature of *Orobanche cumana* populations using the coded triplets system, which is based on the use of eight sunflower lines -termed differentials-, grouped into three sets

Population of <i>O. cumana</i>	Group # 1		Group # 2		Group # 3	
	Differentials	Value if susceptible reaction	Differentials	Value if susceptible reaction	Differentials	Value if susceptible reaction
	AD66 (-)	1	Record ( <i>Or</i> <sub>3</sub> )	1	LC1093 ( <i>Or</i> <sub>6</sub> )	1
	K A-41 ( <i>Or</i> <sub>1</sub> ) <sup>b</sup>	2	S1358 ( <i>Or</i> <sub>4</sub> )	2	P96 ( <i>Or</i> <sub>5</sub> , <i>Or</i> <sub>6</sub> , <i>Or</i> <sub>7</sub> )	2
	J8281 ( <i>Or</i> <sub>2</sub> )	4	P1380 ( <i>Or</i> <sub>5</sub> )	4		
Code <sup>a</sup> :	Total in group # 1		Total in group # 2		Total in group # 3	

<sup>a</sup> Each population is identified by a code of three digits which are obtained by totals due to susceptible reactions of each of the lines into the set. Resistant reactions impart 0. <sup>b</sup> Gene/s governing resistance in each line according to Vrânceanu *et al.* (1980), Akhtouch *et al.* (2002), Pacureanu *et al.* (2004), and Pérez-Vich *et al.* (2004).

propose eight lines of sunflower that can be used as differentials for races of *O. cumana*, notwithstanding the fact that additional ones, showing a consistent resistant reaction to the current most virulent races of the parasite, could be included in the future. The system numerically assigns a value if the differential is susceptible. If the first differential of a set of three is susceptible, it imparts a value of 1; if the second differential is susceptible it imparts a value of 2; and if the third differential is susceptible it imparts a value of 4. The system is additive within each set. When applied to *O. cumana*, the name of each race of the parasite would be a 3-digit code, one digit from each of the three sets of sunflower lines. As an example, if the first and second lines are susceptible, they impart a value of 1+2 (3) or, if the three lines are susceptible, they will impart a value of 1+2+4 (7). If the set only groups two lines, the largest possible value will be 3. This is illustrated in Table 3, where the codes for historical races A to E are given, as well as those for parasite races of a virulence higher than that of E.

Among genotypes identified as being resistant to *O. cumana*, particularly useful as differentials for race characterization are public inbred lines. Because its genetic background is known, public material can easily be exchanged between research groups. In addition to the sunflower genotypes proposed by Vrânceanu *et al.* (1980) as differentials of races A to E of *O. cumana*, other inbred lines were identified by scientists as having a clearly resistant or susceptible discriminating reaction against parasite populations in different countries and/or growing areas within countries. This is the case of line LC1093, resistant to race F in Romania

(Pacureanu-Joita *et al.*, 1998), and line P96 (Fernández-Martínez *et al.*, 2004), which was identified as being resistant to race F from Spain and susceptible to race G from Turkey (Molinero-Ruiz *et al.*, 2014). According to the results of the works by Vrânceanu *et al.* (1980), Pacureanu-Joita *et al.* (1998), Fernández-Martínez *et al.* (2004), Molinero-Ruiz *et al.* (2008), Antonova *et al.* (2013) and Molinero-Ruiz *et al.* (2014), several lines are clear differentials of races of *O. cumana* (Table 2). The use of this single group of differentials worldwide will facilitate a comparison of the results of the characterization of races of *O. cumana* in different countries. Moreover, the most virulent parasite races and their location will be known by scientists working on the achievement of sunflower material with resistance to *O. cumana* in any of the countries where the parasite occurs.

Finally, it is important to note that a diverse genetic composition of *O. cumana* populations was evidenced in Russia and also in Spain (Molinero-Ruiz *et al.*, 2008; Antonova *et al.*, 2013). Genetic heterogeneity within race F populations seems to be a natural and inherent trait, because no components with a virulence higher than F (potential new races) (Molinero-Ruiz *et al.*, 2009) or molecularly distinguishable groups (Molinero-Ruiz *et al.*, 2014), have so far been identified within them. At this moment, genetic studies of the parasite are crucial to bring the knowledge on the parasite to the same level as what known about the genetics of resistance to *O. cumana* in sunflower. Studies on the inheritance of avirulence genes in *O. cumana* have confirmed the gene-for-gene interaction in the *O. cumana*-sunflower parasitic system for races E/F

**Table 3.** Proposal for characterization of populations of *Orobanche cumana* using the coded triplets system, and its correspondence with the traditional method based on the use of consecutive capital letters (A, B, C, etc.) given in chronological order of identification of races

Line of sunflower	Proposed codes for <i>O. cumana</i> races							
	100	300	700	710	730	770	771	773
AD66	S <sup>a</sup>	S	S	S	S	S	S	S
K A-41	R	S	S	S	S	S	S	S
J8281	R	R	S	S	S	S	S	S
Record	R	R	R	S	S	S	S	S
S1358	R	R	R	R	S	S	S	S
P1380	R	R	R	R	R	S	S	S
LC1093	R	R	R	R	R	R	S	S
P96	R	R	R	R	R	R	R	S
Historical race	A	B	C	D	E	F	F or G?	F or G?

<sup>a</sup> S: susceptible, R: resistant.

and the dominant sunflower gene *Or*<sub>5</sub> (Rodríguez-Ojeda *et al.*, 2013), but the inheritance of avirulence genes against other sources of resistance to the most virulent races is still unknown.

## The host *H. annuus*

### Sunflower breeding and genetics of resistance to *O. cumana*

#### *Breeding for resistance to "old" races*

Selection for sunflower resistance to broomrape started in the early 1910s through individual selection methods using open pollinated varieties. The first cultivar resistant to race A, Saratovskij 169, was developed by researchers of the Saratov Experimental Station (Russian Federation) (Plachek, 1921). In the following years, other cultivars with resistance to race A were produced: Kruglik A-41, Zelenka and Fuk-sinka. Some years later Zhdanov (1926), in the Rostov Oblast (Russia), reported the development of several cultivars resistant to race B, in particular Zhdanovsky 6432 and Zhdanovsky 8281 (Table 2). Zhdanov was a pioneer in the use of a species of wild sunflower (*Helianthus tuberosus*) as a source of resistance against *O. cumana* (Vrânceanu *et al.*, 1980; Škorić *et al.*, 2010). During the period 1925-1960, Pustovoit, at the All-Russia Research Institute of Oil Crops (VNIIMK) (Krasnodar, Russian Federation), created highly productive cultivars which were resistant to race B. After the release of these cultivars, the occurrence of new races was not reported for years. As sources of resistance to races A and B were identified, it was also determined that this resistance to the parasite was controlled by dominant genes named *Or* (Burlov & Kostyuk, 1976; Pogorletsky & Geshele, 1976). Some years later a single dominant gene was also found to be responsible for resistance to *O. cumana* of an unknown race from Israel (Ish-Shalom-Gordon *et al.*, 1993). After the identification of race C in Bulgaria and Romania, several genes for resistance to *O. cumana* were introduced into varietal populations of sunflower developed in breeding programmes from Saratov and Krasnodar (Russian Federation), Odessa (Ukraine), Fundulea (Romania) and other places. Extensive research on the parasite was conducted by Vrânceanu *et al.* (1980) in Romania from 1976 to 1980, resulting in the identification of the set of differential lines that had cumulative resistance to the five successive broomrape races A, B, C, D, and E, conferred by the dominant genes *Or*<sub>1</sub>, *Or*<sub>2</sub>, *Or*<sub>3</sub>, *Or*<sub>4</sub> and *Or*<sub>5</sub>, respectively.

#### *Breeding for resistance to race E*

By the end of the 20<sup>th</sup> century, the appearance of new races considerably reduced the available sources of resistance in the germplasm of cultivated sunflower. In Turkey, Gulya *et al.* (1994) found only 22 resistant entries in a field evaluation of 903 accessions. In Spain, Domínguez *et al.* (1996) noted that there was a low frequency of genes for resistance to race E in cultivated sunflower, and after the evaluation of 429 accessions of different origins, only eight resistant entries were identified. In contrast, a high level of resistance was found in wild *Helianthus* spp., mainly in perennial *Helianthus* spp., though resistant annual species were identified as well. Ruso *et al.* (1996) evaluated 24 perennial and 11 annual species for resistance to race E, and identified resistance in most of the perennial and in two annual species: *H. anomalus* and *H. debilis*. In Serbia, Hladni *et al.* (2009) developed five new restorer inbred lines resistant to race E from interspecific populations originating from *H. deserticola*. Genetic resistance to race E was reported in several studies to be controlled by a major dominant gene, *Or*<sub>5</sub> (Sukno *et al.*, 1999; Lu *et al.*, 2000; Pérez-Vich *et al.*, 2004), although other studies indicated genetic control by two independent dominant genes (Domínguez, 1996b). Alonso (1998) noted that the genetic of resistance to *O. cumana* could be more complex than previously thought, suggesting that, in addition to the known major dominant resistant genes, other minor genes may be involved. In fact, molecular studies focused on resistance to race E demonstrated that this resistance was controlled by the major dominant *Or*<sub>5</sub> gene mapping to a terminal, probably the telomeric region, of linkage group 3 (LG3) of the sunflower genetic map (Lu *et al.*, 1999; Tang *et al.*, 2003; Pérez-Vich *et al.*, 2004; Márquez-Lerma *et al.*, 2008), together with a quantitative component determined by four quantitative trait loci (QTL) with a minor effect associated with the number of broomrape shoots per plant (Pérez-Vich *et al.*, 2004). Imerovski *et al.* (2013) demonstrated that simple sequence repeat (SSR) markers of LG3 were also strongly associated with resistance genes *Or*<sub>2</sub>, *Or*<sub>4</sub> and *Or*<sub>6</sub>.

#### *Breeding for resistance to race F*

Later on, sources of resistance to race F were also scarce in germplasm of cultivated sunflower, although valuable resistant germplasm was identified in breeding programmes conducted in Spain (Rodríguez-Ojeda *et al.*, 2001; Fernández-Martínez *et al.*, 2004; Pérez-Vich *et al.*, 2006), Romania (Pacureanu-Joita *et al.*, 2004),

Turkey (Kaya *et al.*, 2009), and Russia (Gontcharov *et al.*, 2004; Gontcharov, 2009). As happened in breeding programmes against race E, wild *Helianthus* spp. proved to be a major reservoir of resistance genes against *O. cumana* race F (Fernández-Martínez *et al.*, 2000), and some of them were incorporated into cultivated sunflower through interspecific hybridization (Jan & Fernández-Martínez, 2002). Resistance to race F from Spain found in the lines KI-534 and P-96, derived from cultivated germplasm, was controlled by recessive alleles at two loci (Rodríguez-Ojeda *et al.*, 2001; Akhtouch *et al.*, 2002), the same recessive genes controlling resistance to race E in the line KI-534 (Rodríguez-Ojeda *et al.*, 2001). Contrarily, resistance to this race from Spain in line J1, derived from interspecific crosses, and to race F from Romania in line LC1093 was found to be mainly dominant and controlled in J1 by the *Or<sub>6</sub>* gene, with incomplete dominance, and *Or<sub>7</sub>*, whose expression was influenced by the environment (Velasco *et al.*, 2007), and in LC1093 by *Or<sub>6</sub>* (Pacureanu-Joita *et al.*, 2004) (Table 2). Although different studies have reported the *Or<sub>6</sub>* and *Or<sub>7</sub>* genes controlling broomrape race F resistance, the allelic relationship between them has not been determined.

QTL analysis of recessive resistance to race F from Spain in line P-96 revealed the presence of six QTL with small to moderate effects on reducing the number of broomrape shoots per plant, three of them being non-race specific (Pérez-Vich *et al.*, 2004). More recently, Louarn *et al.* (2014) identified four QTL for broomrape (Spanish race F) resistance mechanisms in a population derived from the LR1 line, selected for race E resistance from an interspecific genepool (*H. debilis* subsp. *debilis* × *H. annuus*) (Labrousse *et al.*, 2001). Two of the QTL were associated with the number of broomrape tubercles per plant, and two others controlled their necrosis.

#### *Breeding for resistance to O. cumana of virulence higher than F*

Recently, and as previously mentioned, populations overcoming race F resistance, named as races G and H, have also been identified in several countries and a continuous search for sources of resistance for these new races has been carried out (Kaya *et al.*, 2004, 2009; Pacureanu-Joita *et al.*, 2009; Antonova *et al.*, 2013). The non-existence of differential lines for new broomrape races makes defining broomrape races on the global level more difficult (Škorić *et al.*, 2010). Genetic resistance to populations from Romania overcoming race F has been reported to be controlled by two independent dominant genes in line AO-548 (Pacure-

anu-Joita *et al.*, 2008). Recently, resistance to a race classified as G has been transferred from *H. debilis* subsp. *tardiflorus* into cultivated sunflower and identified as being controlled by a single dominant gene (Velasco *et al.*, 2012). In Bulgaria, the outstanding work on interspecific hybridization with several wild *Helianthus* species carried out over decades in the Dobroudja Agricultural Institute (General Toshevo) has produced a number of sources with resistance to races A to G (Christov *et al.*, 1992, 1998, 2009), and lines resistant to race G have been developed from the wild species *H. pauciflorus*, *H. tuberosus*, *H. divaricatus*, *H. hirsutus* and *H. bolanderi* (Christov, 2012). Christov (2013) also succeeded in obtaining sunflower with resistance to *O. cumana* by intergeneric hybridization of *H. annuus* with *Inula helenium* L., *Tithonia rotundifolia* (Mill.) S.F. Blake and *Verbesina helianthoides* Michx. among others. In Serbia, from lines previously selected as being resistant to race E of *O. cumana*, Cvejić *et al.* (2012, 2014) obtained sunflower lines with resistance to races F and G and derived from interspecific crosses with *H. tuberosus* and *H. divaricatus*. Preliminary results of studies of inheritance of resistance to *O. cumana* races higher than F in this material, indicated that the trait is controlled by recessive gene(s) (Cvejić *et al.*, 2014).

As conclusion, up to now, breeding programmes focused on the development of hybrids of sunflower carrying resistance to *O. cumana* are mainly based on single dominant *Or* genes. To ensure their success, the best way to proceed is to pick out an elite line and cross it with a source of *Or* genes, which should then be incorporated into the breeding material using backcross breeding (recurrent cross-breeding together with screening for resistance in all BC generations). At the start of the programme, the breeder has to determine which race or races are present in the region for which the hybrids are being developed. However, alternative breeding strategies are required to increase the durability of genetic resistance to *O. cumana*. The most significant results are achieved by interspecific hybridization in which wild species of genus *Helianthus* are used as donors of the gene of resistance. Transferring resistance genes from perennial species is generally difficult, due to problems associated with early hybrid embryo abortion and sterility in F<sub>1</sub> and BC<sub>1</sub>F<sub>1</sub> generations. This problem can be overcome by using embryo rescue and chromosome doubling of the F<sub>1</sub> (Jan & Fernández-Martínez, 2002). Also, alternative breeding strategies involving vertical resistance should incorporate gene pyramiding, alternation of several forms of a hybrid with different *Or* genes, or mixtures of these different forms grown together. Finally, to achieve the best use of these major genes, they need to be backed-up by

quantitative, non-race specific resistance (Pérez-Vich *et al.*, 2004; 2006). These strategies will require QTL analysis and development of molecular markers linked to major and minor resistance genes to ensure that they are simultaneously introgressed during backcross, and a detailed characterization of the physiological mechanisms underlying genetic resistance.

### Mechanisms of resistance against *O. cumana*

One of the most important aspects when selecting for broomrape resistance is to obtain information on the physiological basis of the different resistance sources, with the ultimate goals of conducting physiology-based breeding and pyramiding resistance genes underlying different resistance mechanisms (Pérez-Vich *et al.*, 2013). The mechanisms of sunflower resistance to *O. cumana* have been studied for a long time. Early studies already mentioned the importance of the root system's pH for broomrape resistance in sunflower. Morozov (1947) cited the findings of Rihter (1924), according to which low pH values in the root of the host plant promote susceptibility to this parasite. On the contrary, basic soils (pH  $\text{Cl}_2\text{Ca}$  8.14) have been related to a lower number of broomrape stems per sunflower plant as compared to acid soils (pH  $\text{Cl}_2\text{Ca}$  6.17) in Spain (Lozano-Cabello, 1999).

Defensive responses such as physical or chemical barriers that prevent parasite intrusion and development can be activated at three different stages of the parasite-host interaction: pre-attachment of the seedlings, pre-haustorial stage, or post-haustorial stage (Pérez-de Luque *et al.*, 2009). Results obtained by Morozov (1947) established that sunflower root cells contain substances which activate germination of broomrape seeds and in that way support the development of a sprout (stimulators). Long after that, Alonso (1998), Wegmann (1998), Matusova *et al.* (2004) and Höniges *et al.* (2008) also pointed out the importance of broomrape germination stimulants (see 'Overview of its biology and genetics' section for more details). Low exudation of germination stimulants by sunflower roots has been described as a pre-attachment resistance mechanism to race E in the sunflower line LR1, derived from *H. debilis* subsp. *debilis* (Labrousse *et al.*, 2001). Another pre-attachment resistance mechanism is the exudation by sunflower roots of seed germination inhibitors and/or inhibitors of radicle exoenzymes (Höniges *et al.*, 2008). Phytoalexins (compounds of a phenolic nature), in particular 7-hydroxylated simple coumarins, have been suggested as playing a defensive role by preventing broomrape germination and subsequent

connection with sunflower roots in the sunflower variety Cortés (Eurosemillas, Córdoba, Spain) resistant to Spanish *O. cumana* populations collected between 1994 and 1996 (Serghini *et al.*, 2001).

Pre-haustorial resistance mechanisms have also been reported in the sunflower-*O. cumana* interaction. These take place from the first contact of the parasite seedling with the host root up to the establishment of vascular connections through haustorium development (Pérez-de Luque *et al.*, 2009). Studies in resistant *H. annuus* (Dörr *et al.*, 1994) and *H. debilis* (Labrousse *et al.*, 2001) have identified the formation of an encapsulation layer that prevents intrusion of the broomrape radicle into the cortex. Physical barriers reinforcing host cortical cell walls through suberization and protein cross-linking (in cv. HE-39999, resistant to race F, Advanta Ibérica, Sevilla, Spain) (Echevarría-Zomeño *et al.*, 2006), or callose depositions (in line LR1, resistant to race E) (Letousey *et al.*, 2007) have been reported. In this last case, callose accumulation around the parasite penetration site was correlated with the induction of a callose synthase gene (*HaGSL1*) (Letousey *et al.*, 2007). Accumulation of lignin and its precompounds in injured host cells has also been described in resistant sunflower genotypes (Panchenko & Antonova, 1975; Antonova, 1994; Jorrín *et al.*, 1996). Lignification and suberization for cell wall fortification are processes linked to the activity of peroxidases (Echevarría-Zomeño *et al.*, 2006), and the important role of these enzymes in relation to resistance to *O. cumana* was reported in early studies in sunflower by Suhorukov (1930) (cited by Morozov, 1947) and later by Antonova & ter Borg (1996). Chemical barriers have also been described in the above-mentioned sunflower genotype HA-39999 (resistant to race F), and again phenolics appear as toxic compounds in the form of accumulation and excretion of phytoalexins into the apoplast that create a toxic environment around the broomrape infection point (Echevarría-Zomeño *et al.*, 2006).

Once the parasite develops vascular connections to the host, the parasitic phase begins and defence mechanisms are post-haustorial (Pérez-de Luque *et al.*, 2009). These mechanisms are characterized by the death of broomrape tubercles during their initial developmental stages. A physical barrier consisting of sealing sunflower xylem vessels with gel or gum-like substances which block the flux of water and nutrients from the host to the parasite, has been observed in the resistant line LR1 against race E (Labrousse *et al.*, 2001). As can be concluded for LR1 or HA-39999 genotypes, several resistance mechanisms occurring at different stages may operate within a single resistant genotype.

## Genetic resistance to herbicides as a breeding alternative for the control of *O. cumana*

During the last decade another alternative for the control of *O. cumana*, based on the development of herbicide resistance in sunflower (Sala *et al.*, 2012a) together with herbicide treatments, has been implemented. This option is generally used in combination with the available *O. cumana* resistance genes. The development of sunflower genotypes bearing an aceto-hydroxyacid synthase (AHAS, EC 4.1.3.18, also known as acetolactate synthase) mutant gene (Shaner *et al.*, 1984; Ray, 1984), and therefore resistant to AHAS, has made it possible to successfully control broomrape regardless of the race composition of the populations of these weeds (Škorić & Pacureanu, 2010). At present, three different herbicidal technologies commercially available are based on the introduction of different AHAS alleles conferring resistance to particular AHAS inhibitors in the crop (Sala *et al.*, 2012a).

The reduction in herbicide binding is caused by mutations at key sites in the genes coding for the catalytic subunit of AHAS. Several authors have reviewed known mutations of the AHAS genes that confer resistance to AHAS-inhibiting herbicides in weeds and crops (Preston & Mallory-Smith, 2001; Tranel & Wright, 2002; Tan *et al.*, 2005). Based on molecular studies, Kolkman *et al.* (2004) identified and characterized three genes coding for the AHAS catalytic subunits in sunflower (*Ahas11*, *Ahas12* and *Ahas13*). *Ahas11* is a multiallelic locus and the only member of this small family in which all the induced and natural mutations for herbicide resistance have been described thus far in sunflower. *Ahas11-1*, also known as *Imr1* (Bruniard & Miller, 2001) or *Ar<sub>pur</sub>* (Kolkman *et al.*, 2004), harbours a C-to-T mutation in codon 205 (*Arabidopsis thaliana* nomenclature), which confers a moderate resistance to imidazolinone (IMI). *Ahas11-2* (also known as *Ar<sub>kan</sub>*) shows a C-to-T mutation in codon 197 conferring high levels of sulfonylurea (SU)-resistance (Kolkman *et al.*, 2004). *Ahas11-3* presents a G-to-A mutation in codon 122, which confers high levels of IMI-resistance (Sala *et al.*, 2008a), and *Ahas11-4* harbours a G-to-T mutation in codon 574, which endows a wide range of resistances to four families of herbicides targeting AHAS (Sala & Bulos, 2011).

Imidazolinone and sulfonylurea herbicides have been seen to have a broad spectrum of weed control activity, flexibility in timing of application, low usage rates, and low mammalian toxicity (Brown, 1990; Tan *et al.*, 2005). These herbicides inhibit the enzymatic activity of AHAS, the first enzyme in the pathway for the synthesis of the branched chain amino acids valine, leucine, and isoleucine (Singh, 1999). This same en-

zyme has been shown to be the site of action for the triazolopyrimidines (TZ, Subramanian & Gerwick, 1989), pyrimidylxybenzoates (POB, Subramanian *et al.*, 1990), and sulfonylaminocarbonyl-triazolinones (Santel *et al.*, 1999). Because this technology depends on the availability of IMI- and SU-resistant hybrid cultivars, the development of IMI- or SU-resistant plants with altered AHAS genes and enzymes is imperative. Herbicide resistant (HR) plants have been discovered in sunflower, which permit the development and commercialization of at least four HR traits. Resistance in these traits is due to a form of the AHAS large subunit enzyme (AHASL) that is less sensitive to herbicide inhibition and is conferred by a single, partially dominant nuclear gene (Sala *et al.*, 2012a).

The use of IMI-resistant sunflowers (also termed as Imisun) for the control of *O. cumana* was early advised by Alonso *et al.* (1998). The genetics behind this trait was well described (Bruniard & Miller, 2001). The first IMI-resistant trait in sunflower was developed by the BASF Company and in simultaneous use with IMI herbicides was called Clearfield technology (Tan *et al.*, 2005). A set of different Clearfield herbicides were registered to be applied as post-emergence treatments in sunflower. These herbicides were formulated in several ways, using different imidazolinone molecules, and taking into account the specific characteristics of the weeds, soils and crop rotations in each sunflower production region. It is important to note that the moment of application is crucial for an effective control of *O. cumana*. Pulsar® (imazamox 40 g/L) was effective in split applications between the plant stages of 2 and 4 true leaves (Demurin & Perstenyeva, 2010; Masirevic *et al.*, 2010). Due to the simultaneous control of both *O. cumana* and key weeds, the Clearfield technology for sunflower production reached about 50% market share in Trakya Region (European part of Turkey) and over 70% of the Turkish sunflower area in the last years (Kaya & Evci, 2009).

The second IMI-resistant trait in sunflower, known as CLPlus®, is controlled by the expression of the partially dominant nuclear allele *Ahas11-3*, which was developed by seed mutagenesis and selection with imazapyr (Sala *et al.*, 2008a). Based on a vast array of environmental conditions and on biochemical studies, it was determined that the CLPlus® trait affords greater resistance to IMI herbicides than the Imisun trait, also providing a better stability to cope with the unpredictable portion of the environmental variation (Sala *et al.*, 2008a,b,c; 2012b). In fact, the CLPlus® trait displays the lowest level of inhibition of the AHAS enzyme extracts by imidazolinones, which results in a higher level of accumulation of biomass after imidazolinone application at the above-ground (Sala *et al.*, 2012a) and root levels (Sala *et al.*, 2012b).

SU-resistant and ExpressSun® sunflowers, both with resistance to SU herbicides, were developed separately from wild sunflower populations discovered in the USA (Al-Khatib *et al.*, 1999) and by ethyl methanesulfonate mutagenesis over the line HA89 (Gabard & Huby, 2001), respectively. The resistance allele *Ahas11-2* from SU-resistant sunflowers was introgressed into cultivated sunflower by forward crossing and selection with the herbicide tribenuron, and gave rise to the trait known as Sures (Miller & Al-Khatib, 2004). Even though the inheritance of these traits has not been reported, it is well established that the target-site-resistance is the result of the mutation P197L at the *Ahas11* locus (Kolkman *et al.*, 2004), and that damage differences between Sures-resistant breeding lines are the result of the presence of modifier genes (Miller & Zollinger, 2004). These mutations were independently used by some public institutions and by private companies to develop SU-resistant hybrid cultivars in many countries, increasing the range of available herbicides in sunflower (Jocić *et al.*, 2011; Streit, 2012). The use of SU herbicides for the control of *O. cumana* in SU-resistant sunflowers was tested under field conditions. A split post-emergence application of metsulfuron methyl provided a good level of control and was not phytotoxic to sunflower (Gabard & Huby, 2001). When the response of one SU- and one IMI-resistant sunflower hybrids to seven doses of imazamox and tribenuron methyl was analysed in-field and in greenhouse experiments, imazamox proved to be more effective for the control of the parasite in IMI-resistant sunflower than tribenuron in SU-resistant sunflowers (Malidza *et al.*, 2012).

Finally, a novel HR trait in sunflowers, still under development, is known as AIR. It is controlled by the *Ahas11-4* and presents a completely new pattern of cross-resistance for sunflower, since it shows a broad range level of resistance to different AHAS-inhibiting herbicides (IMI, SU, TZ and POB). Furthermore, this allele also presents a higher level of resistance to IMI and SU than lines carrying the Imisun and the Sures traits, respectively (Sala & Bulos, 2011). It is known that sunflower lines developed to cope with some AHAS-inhibiting herbicides are susceptible to foliar applications and, in many cases, to soil residues of other AHAS-inhibiting herbicides (Howatt & Endress, 2006). In these cases, the cross-resistance of *Ahas11-4* could allow sunflower hybrids carrying this allele to cope with the soil residues of other types of AHAS-inhibiting herbicides from the fallow or the previous crop.

Other candidate genes are now under study aiming at the development (Sala *et al.*, 2008a; Sala & Bulos, 2011) and characterization (Sala *et al.*, 2008b) of new

HR traits, which could lead to the formulation of new herbicides, targeting different enzymes. Similarly, herbicide formulations and seed dressing technologies using different AHAS-inhibiting herbicides and which have proven to be useful for the control of *Striga* spp. in corn and sorghum (Tuinstra *et al.*, 2009; Ransom *et al.*, 2012), should be tested over the known HR sunflower technologies in order to provide new strategies for the control of *O. cumana*.

Other chemical options have a detrimental effect against *O. cumana*, such as inducers of seed germination leading to suicidal germination of the parasite in the absence of sunflower (Lachia *et al.*, 2014), or inhibitors of the germination process (Okazawa & Benesh, 2011) but, as they are not coupled with genetic breeding of sunflower for traits of resistance to chemicals, they will not be covered in this review. Extensive information on chemical signals from hosts and their effect on parasite species can be found in other works (Smith *et al.*, 1990; Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008; Yoneyama *et al.*, 2008). Finally, the availability of next-generation sequencing technologies, metabolomics and its applications to produce continuous and massive information about parasitic weeds (Piednöl *et al.*, 2012; Westwood *et al.*, 2012; Pineda-Martos *et al.*, 2014) should be exploited. Previously known herbicide target genes obtained from model plants can be used for a rapid and effective characterization using different bioinformatics approaches on the creation of new herbicides specifically directed at *O. cumana*. The discovery of differences in metabolic pathways between this organism and sunflower will be vital for these developments. Also, the sequences generated will provide insights into the biology of parasitism and advance progress towards understanding parasite virulence.

In summary, the parasitic weed *O. cumana* poses a risk to sunflower oil production in countries of Southern and Eastern Europe every year, and causes, on average, 50% sunflower seed losses when susceptible hybrids are grown. Understanding the biology of the interaction between the parasite and its host is a necessary step toward the development of selective control methods. Physiological and molecular mechanisms governing the *Orobanchae* development and the establishment of the interaction with its hosts, for example the chemical stimulation of parasite seed germination, or the role of degrading enzymes on the parasite progression between host cells, have been deeply studied and characterized. However, much remains to be done on deciphering the biological processes that are particular to *O. cumana* in sunflower. This knowledge would lead to the identification of vulnerabilities as specific targets for new control methods. Breeding for

resistance is a recurrent, feasible and effective alternative for controlling *O. cumana*. Different races of the parasite have been identified in all the countries where it threatens sunflower oil production and, at the same time, important breeding works have been devoted to the search for effective genetic resistance against the increasingly virulent local parasite populations. Since information on pathogenic traits of the parasite is obtained locally, its validity is restricted to particular geographical areas within Europe. Comparisons of results giving the characterization of races at an international level, as well as the effectiveness of resistance sources in international sunflower breeding programmes, will be favoured by the universal adoption of the coded triplets system for nomenclature of *O. cumana* races. On the other hand, breeding sunflowers for resistance to AHAS-inhibiting herbicides has appeared during the past decade as another alternative for the control of *O. cumana* together with other weeds of the crop, and irrespective of parasite races. IMI- and SU- resistant plants were discovered in sunflower, which permitted the development and commercialization of four herbicide resistant traits. This alternative for the control of *O. cumana* is, in some instances, implemented together with genetic resistance to races of the parasite, and is likely to undergo an important development as long as novel HR traits are discovered.

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